## REMARKS/ARGUMENTS

### The Status of the Claims.

Claims 1-15 and 73-93 are pending with entry of this amendment, claims 16-72 being cancelled and claims 73-93 being added herein. Claims 1, 2, 5-12, 14 and 15 are amended herein. These amendments introduce no new matter and support is replete throughout the specification. These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter or agreement with any objection or rejection of record.

Claims 1and 2 were amended to correct typographical errors and to better clarify the claimed invention; support for the amendments can be found throughout the specification, at, for example, paragraphs [0008], [0055] to [0056], [0061] to [0117], and in the Figures, the Examples, and claims as originally filed. With respect to claim 5, support for an antibody can be found in the specification at, for example, [0009], [0033] to [0035], [0140], and in original claims 12 and 52. With respect to claims 6 and 7, support for the combinatorial library embodiments can be found in the specification at, for example, paragraphs [0008] and [0013]. Claims 8 and 9 were amended to correct typographical errors. With respect to claim 10, support for the use of spin labels and magnetic beads as reporter moieties can be found in the specification at, for example, paragraphs [0080], [0083], and [0086], and in the original claims. With respect to claim 11, support for iminodiacetic acid (IDA) lipid can be found in the specification at, for example, paragraphs [0086], and [0096] to [0102] and in original claims 27, 41 and 53. With respect to claim 12, support for protein G can be found in the specification at, for example, [0025], [0140], and original claims 28, 42, and 52. With respect to claims 14 and15, support for identifying the ligand can be found in the specification at, for example, [0007] to [0008], [0056] to [0057], [0118] to [0120] and in the original claims.

With respect to claims 73-76, support for the metal chelation can be found in the specification at, for example, paragraphs [0009], [0025], [0099] to [0103] and [0111]. With respect to claims 77-78, support for the ligand-reporter contact can be found in the specification at, for example, paragraphs [0008], [0055], [0109], [0128] to [0130], and [0151]. With respect to claim 79, support for microtiter plates can be found in the specification at, for example, paragraphs [0128] to [0131] and Example 1. With respect to claims 80-84, support for the detecting step can be found in the specification at, for example, paragraphs [0114] to [0123]. With respect to claim 85, support for the detecting steps can be found in the specification at, for example, paragraph [0124]. With respect to claim 86, support for contacting the cell with at least two different ligands can be found in the specification at, for example, paragraphs [0062] to [0063] and [0129]. With respect to claims 87-93,

support for using a plurality of cells can be found in the specification at, for example, [0107] to [0110], [0117], [0121] to [0131], and in the Examples.

Applicants submit that no new matter has been added to the application by way of the above Amendment. Accordingly, entry of the Amendment is respectfully requested.

**The Election/Restriction Requirement.**

Pursuant to a restriction requirement made final, Applicants cancel claims 16-72 with entry of this amendment. Please note, however, that Applicants reserve the right to file subsequent applications claiming the canceled subject matter and the claim cancellations should not be construed as public dedication, abandonment or agreement with the Examiner's position in the Office Action.

**The Information Disclosure Statement.**

Applicants note with appreciation the Examiner's consideration of the references cited in the Information Disclosure Statements (Form 1449) submitted on December 12, 2002 and July 7, 2003. In response to the Examiner's observation regarding the missing Matzku et al. reference, Applicants respectfully provide a supplemental Form 1449 and an additional copy of the cited reference for consideration.

**Objection to Claim 2**

Claim 2 was objected to under 37 CFR 1.75(c) as allegedly being of improper dependent form. Applicants have amended claims 1 and 2 and added new claims 77-78 to further clarify the claimed subject matter, and respectfully request that the objection be withdrawn.

**35 U.S.C. §112, Second Paragraph.**

Claims 1-3, 5-10 and 12-15 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject invention. Applicants traverse to the extent that the rejections apply to the claims as amended, and respectfully request that the rejections be withdrawn.

Claim 1 as amended distinctly claims the subject invention

In paragraph 9 of the Office Action, the Office requested clarification of the language of claim 1, particularly with respect to the method steps involving the reporter. Applicants respectfully thank the Examiner for the helpful suggestions in clarifying the claimed invention.

Claim 1 as amended is drawn to methods of detecting ligand internalization into a cell, which methods include the steps of i) contacting said cell with a ligand and a reporter, wherein

the reporter non-covalently couples to the ligand; ii) dissociating the reporter from non-internalized ligand and removing dissociated reporter from the surface of said cell; and iii) detecting the presence of the reporter remaining in the cell, whereby the presence of the reporter indicates that said ligand is internalized into the cell. Applicants submit that the claim as amended distinctly claims the subject invention, and respectfully request that the rejection be withdrawn.

Clarification of the step of "contacting the cell"

In paragraph 10 of the Action, the Office rejected claims 1-3 and 5-10 under 35 U.S.C. §112, second paragraph, as allegedly indefinite with respect to the step of contacting the cell with the ligand and the reporter. Applicants have amended claims 1 and 2, and provided new claims 77-78 to further clarify the claimed subject matter and rectify the appearance of the allegedly contradictory language in claims 1 and 2. As elucidated in the amended and newly presented claims, the reporter can be noncovalently coupled to the ligand before, after, or concomitant with association of the ligand with the cell. Applicants submit that the amended claims are not indefinite with respect to the contacting step, and respectfully request that the rejection be withdrawn.

Antecedent basis for the term "epitope tag"

Claim 12 was rejected under 35 U.S.C. §112, second paragraph, as indefinite with respect to the antecedent basis of the term "said epitope tag." Applicants thank the Examiner for pointing out the inadvertent error in antecedent basis, and respectfully request that the rejection be withdrawn in light of the amended claim.

**35 U.S.C. §102**

THE CLAIMS ARE NOT ANTICIPATED BY USPN 6,794,128 TO MARKS ET AL.

Claims 1, 3, 5-7, 10, 13 and 14 were rejected under 35 U.S.C. §102(e) as allegedly anticipated by US Patent No. 6,794,128 to Marks et al. Applicants traverse.

In order for a reference to anticipate an invention, the reference must teach each and every element of the claimed invention. Claim 1 is drawn to methods of detecting ligand internalization through the use of a reporter non-covalently coupled to a ligand. The methods include the steps of i) contacting said cell with a ligand and a reporter, wherein the reporter non-covalently couples to the ligand; ii) dissociating the reporter from non-internalized ligand and removing dissociated reporter from the surface of said cell; and iii) detecting the presence of the reporter remaining in said cell, whereby the presence of the reporter indicates that said ligand is internalized into said cell.

Marks is alleged to teach the methods of the subject invention. However, the Office has not specified how the cited art teaches or discloses the step of dissociating the reporter from the ligand and removing dissociated reporter from the cell surface. Applicants submit that by not showing how the reference teaches every element of the claimed invention, the Office has not made out a *prima facia* case for anticipation. As such, the rejection is improper and should be withdrawn.

**35 U.S.C. §103(a)**

THE CLAIMS ARE PATENTABLE OVER HEIN, HURWITZ AND RAKOWITZ-SZULCZYNSKA

Claims 1, 3, 10, and 13-15 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Hein et al (USPN 6,251,392) in view of Hurwitz et al. (1995 Proc. Acad. Natl. Sci. USA 92:3353-3357) and Rakowitz-Szulczynska et al. (USPN 5,296,348). Applicants traverse.

Three requirements must be met for a *prima facie* case of obviousness. First, the prior art reference must teach all of the limitations of the claims. M.P.E.P § 2143.03. Second, there must be a motivation to modify the reference or combine the teachings to produce the claimed invention. M.P.E.P. § 2143.01. Third, a reasonable expectation of success is required. M.P.E.P. § 2143.02. The teaching or suggestion to combine and the expectation of success must be both found in the prior art and not based on Applicants' disclosure. M.P.E.P. §2143.

Hein does not teach the limitations of the claims

Claim 1 is drawn to methods of detecting ligand internalization into a cell. The claimed methods include the steps of i) contacting said cell with a ligand and a reporter, wherein the reporter non-covalently couples to the ligand; ii) dissociating the reporter from non-internalized ligand and removing dissociated reporter from the surface of said cell; and iii) detecting the presence of the reporter remaining in said cell, whereby the presence of the reporter indicates that said ligand is internalized into said cell. Claim 3 is drawn to methods involving ligand binding to cell surface receptors, claim 10 is drawn to various reporter embodiments, claim 13 is drawn to methods involving cancer cells, and claims 14-15 are drawn to an optional step of identifying the internalized ligand.

Hein is alleged to teach methods of identifying internalizing ligands through conjugation of the ligand to a reporter, exposing the conjugate to a cell, and detecting the reporter inside the cell. However, as noted at page 8 of the Office Action, Hein does not teach the step of dissociating the reporter from non-internalized ligand and removing dissociated reporter from the surface of said cell. Thus, Hein does not teach all of the limitations of the claimed invention.

Hurwitz and Rakowitz-Szulczynska do not remedy the deficits in Hein

Hurwitz and Rakowitz-Szulczynska are also alleged to teach methods for identification of internalizing antibodies. However, neither reference remedies the deficits in Hein. For example, Hurwitz and Rakowitz-Szulczynska describe removing non-internalized antibodies from the cell surface, but neither publication teaches or discloses the step of dissociating the reporter from the non-internalized antibody and removing the dissociated reporter from the cell surface. To the contrary, the reporters employed by Hurwitz and Rakowitz-Szulczynska cannot be dissociated from the associated ligand. Hurwitz teaches radiolabeling of monoclonal antibodies (ligands) with $^{125}I$ or $^{35}S$, and Rakowitz-Szulczynska discloses $^{125}I$ -labeled monoclonal antibodies. In both cases, the radiolabel is covalently associated with the ligand (e.g., the tyrosine residues are halogenated using $^{125}I$, or the proteins are synthesized using $^{35}S$-methionine) and cannot be dissociated from the ligand and removed from the cell surface as provided in the methods of the subject invention.

Since Hein does not teach the step of dissociating the reporter from non-internalized ligand, and this deficit is not remedied by either Hurwitz or Rakowitz-Szulczynska, the first criteria for a *prima facie* case of obviousness has not been met. Applicants submit that the rejection is improper and respectfully request that it be withdrawn.

There is no motivation to combine the cited publications or reasonable expectation of success

When a rejection depends on a combination of prior art references, there must be some teaching, suggestion, or motivation to combine the references. *In re Geiger,* 815 2 USPQ2d 1276, 1278 (Fed. Cir. 1987). Moreover, to support an obviousness rejection, the cited references must additionally provide a reasonable expectation of success. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991), *citing In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). Modification unwarranted by the disclosure of a reference is unfounded (Carl Schenck A.G. v. Nortron Corp., 713 F.2d 782, 218 USPQ 698, 702, Fe, Cir. 1983).

Given that the combination of references cited by the Examiner fail to teach or suggest all of the elements of the presently claimed invention, Applicants submit that there would not be any mechanism or motivation to combine the cited publications and arrive at the methods of the subject invention, nor would there be any reasonable expectation of success. For example, since the radiolabel reporters employed in the references cannot be dissociated from the antibody ligand, one would not consider, much less expect to succeed with, a step of dissociating the reporter from non-internalized ligand and removing dissociated reporter from the surface of said cell.

The prior art must suggest the desirability of the modification

The Office alleges that removal of antibodies from the cell surface by a washing step "would also inherently result in the dissociation of the reporter from the antibody where the reporter and antibody are non-covalently bound." (See page 9). The Office goes on to state that "it is clear from the references that the reporter is the moiety being detected, it would also have been obvious to those in the art that the internalized [reporter] inside the cell could be distinguished from those merely bound to the surface by dissociating the ligand on surface-bound antibodies..." citing Tsaltas et al. (1993 Immunol. Invest. 22:1-12). Applicants traverse.

The mere fact that the prior art may be modified in the manner suggested by the Office (i.e., by substituting non-covalently attached reporter moieties for the covalently radiolabeled species provided by Hurwitz, and claiming that the reporters are then dissociated from the ligand during a putative washing step to remove ligand from a cell surface) does not make the modification obvious unless the prior art suggests the desirability of the modification (In re Fritch, 972 F.2d 1260, 23 USPQ 2d 1780, 1783, Fed. Cir. 1992). Applicants note that the title of the Tsaltas reference is "Cell Membrane Antigen-Antibody Complex Dissociation by the Widely Used Glycine-HCl method: An Unreliable Procedure for Studying Antibody Internalization" (emphasis added). Furthermore, the pages cited in Tsaltas discuss how the methods described therein are generally ineffective for antibody internalization studies (second and third paragraphs on page 8). Applicants submit that Tsaltas actually teaches away from the present invention, by indicating that the low pH conditions typically used in the art would "introduce major inaccuracies in such assays" and "would generally prove ineffective in fully dissociating [antigen-antibody] bonds." Applicants submit that the combination of Tsaltas with the other references cited in the rejection do not teach the limitations of the claimed invention, nor do they (alone or in combination) provide any mechanism or motivation to combine the cited publications, provide any reasonable expectation of success, or otherwise suggests the desirability of modifying the cited references to arrive at the methods of the subject invention.

Since the criteria for a *prima facie* case of obviousness have not been met. Applicants submit that the rejection is improper and respectfully request that it be withdrawn.

## THE CLAIMS ARE PATENTABLE OVER HEIN, HURWITZ, AND RAKOWITZ-SZULCZYNSKA IN FURTHER VIEW OF BURMER, COLLINS AND FREED

Claims 2 and 8 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Hein et al. in view of Hurwitz et al. and Rakowitz-Szulczynska et al., and in further view of Burmer et al (USPN 6,087,103) and Collins (USPN 5,770,422) and Freed (USPN 5,597,719). Applicants traverse.

Claim 2 is drawn to methods for detecting ligand internalization involving contacting the cell with a ligand comprising an epitope tag; and contacting the ligand with a reporter comprising a moiety that binds said epitope tag. The step of contacting the ligand with the reporter can be performed prior to, concomitant with, or after the step of contacting the cell with the ligand, as clarified in newly submitted claims 77-78. Claim 8 is drawn to methods for detecting ligand internalization involving reporters non-covalently coupled to the ligand via an epitope tag.

The first requirements that must be met for proving a *prima facie* case of obviousness is that the prior art reference teach all of the limitations of the claims. As noted above, neither Hein nor Hurwitz nor Rakowitz-Szulczynska teach the step of dissociating the non-covalently-associated reporter from non-internalized ligand and removing dissociated reporter from the surface of said cell. Burmer is alleged to teach methods for identification of target-ligand interactions as well as means of conjugating labels to ligands. However, Burmer does not teach the use of epitope tags (as acknowledged in the Office Action), nor does Burmer teach or disclose methods involving dissociating the reporter from non-internalized ligand and removing dissociated reporter from the cell surface. Collins and Freed are alleged to teach means for detection of proteins in the art, including the use of epitope tags, but they also do not teach dissociating the reporter from non-internalized ligand and removing dissociated reporter from the cell surface. Since the cited references do not teach or disclose all of the limitations of the claimed invention, the first criteria for proving a *prima facie* case of obviousness has not been met. Applicants submit that the rejection is thus improper and respectfully request that it be withdrawn.

## THE CLAIMS ARE PATENTABLE OVER HEIN, HURWITZ, AND RAKOWITZ-SZULCZYNSKA IN FURTHER VIEW OF BARBAS AND WARD

Claims 6 and 7 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Hein et al. in view of Hurwitz et al. and Rakowitz-Szulczynska et al., and in further view of Barbas et al. (Proc. Acad. Natl. Sci. USA 88:7978-7982) and Ward et al. (J. Immunol. Methods 189:73-82). Applicants traverse.

Claims 6 and 7, as amended, are drawn to methods for detecting ligand internalization involving ligands from combinatorial libraries. The methods include the step of dissociating the reporter from non-internalized ligand and removing dissociated reporter from the surface of said cell. As noted above, neither Hein nor Hurwitz nor Rakowitz-Szulczynska teach the step of dissociating the non-covalently-associated reporter from non-internalized ligand and removing dissociated reporter from the surface of the cell. Barbas is alleged to teach production of phage display libraries that express antibodies; Ward is alleged to teach isolation of antibodies from libraries

through the use of an enzymatic cleavage site. However, neither of the cited publications teaches the step of dissociating the non-covalently-associated reporter from non-internalized ligand and removing dissociated reporter from the cell surface. Since the cited art does not teach or disclose all the limitations of the claimed invention (the first criteria for proving a *prima facie* case of obviousness), Applicants submit that the rejection is improper and respectfully request that it be withdrawn.

THE CLAIMS ARE PATENTABLE OVER HEIN, HURWITZ, AND RAKOWITZ-SZULCZYNSKA IN FURTHER VIEW OF PLANT AND SZOKA

Claims 9-11 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Hein et al. in view of Hurwitz et al. and Rakowitz-Szulczynska et al., and in further view of Plant et al. (USPN 5,389,523) and Szoka et al. (USPN 6,593,308). Applicants traverse.

Claims 9-11 are drawn to methods involving specified reporters and/or ligands. These methods include the step of dissociating the reporter from non-internalized ligand and removing dissociated reporter from the surface of said cell. As noted above, neither Hein nor Hurwitz nor Rakowitz-Szulczynska teach this step. Plant is alleged to teach coupling of peptides to liposomes and detection thereof; Szoka is alleged to teach drug delivery compositions involving liposomes. However, none of the cited publications teaches the step of dissociating the non-covalently-associated reporter from non-internalized ligand and removing dissociated reporter from the cell surface. Since the cited art does not teach or disclose all the limitations of the claimed invention (the first criteria for proving a *prima facie* case of obviousness), Applicants submit that the rejection is improper and respectfully request that it be withdrawn.

THE CLAIMS ARE PATENTABLE OVER HEIN, HURWITZ, AND RAKOWITZ-SZULCZYNSKA IN FURTHER VIEW OF STEWART

Claims 9 and 12 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Hein et al. in view of Hurwitz et al. and Rakowitz-Szulczynska et al., and in further view of Stewart et al. (USPN 6,087,452). Applicants traverse.

Claims 9 and 12 are drawn to methods involving ligands having epitope tags (claim 9) and ligand embodiments employing epitope tags coupled via protein A (claim 12). These methods include the step of dissociating the reporter from non-internalized ligand and removing dissociated reporter from the surface of said cell. As noted above, neither Hein nor Hurwitz nor Rakowitz-Szulczynska teach this step. Stewart is alleged to teach the use of polyhistidine/protein A for attachment of antibodies to another compound. However, Stewart does not teach the step of dissociating the non-covalently-associated reporter from non-internalized ligand and removing

dissociated reporter from the cell surface. Since the cited art does not teach or disclose the limitations of the claimed invention (the first criteria for proving a *prima facie* case of obviousness), Applicants submit that the rejection is improper and respectfully request that it be withdrawn.

## Double Patenting

The claims are patentable over USPN 6,794,128

Claims 1, 3, 10, 13 and 14 were rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-7, 9-12, 23-31 and 33-36 of USPN 6,794,128 to Marks et al. (the '128 patent). The Office asserts that the pending claims of the subject application are allegedly generic to the claims in the '128 patent. Applicants traverse.

The claims of the subject invention are drawn to methods of detecting ligand internalization into a cell, and include the steps of i) contacting said cell with a ligand and a reporter, wherein the reporter non-covalently couples to the ligand; ii) dissociating the reporter from non-internalized ligand and removing dissociated reporter from the surface of said cell; and iii) detecting the presence of the reporter remaining in said cell, whereby the presence of the reporter indicates that said ligand is internalized into said cell.

Claims 1-7, 9-12, 23-31 and 33-36 of the cited patent are drawn to methods of selecting a polypeptide that is internalized into a target cell, including the steps of contacting one or more target cells with one or more members of a phage display library displaying one or more polypeptides and identifying internalized library members. The methods of claims 1-7 and 9-12 also include the step of culturing the target cells and enriching the internalized library members by at least 30-fold as compared to on-internalized library members. The methods of claims 23-31 and 33-36 also include the steps of culturing the target cells under conditions wherein members of said phage display library bound to an internalizing marker become internalized, and reducing non-internalized library members by removing phage trapped in an extracellular matrix.

Applicants submit that the rejection under the judicially created doctrine of obviousness-type double patenting is improper. The Office has not established how claims 1-7, 9-12, 23-31 and 33-36 of the cited patent provide for methods in which a reporter noncovalently coupled to a ligand is dissociated from non-internalized ligand and removed from the surface of a cell. Applicants respectfully request that the Office withdraw the improper rejection.

The claims are patentable over USSN 10/855,755

Claims 1, 3, 10, 13 and 14 were provisionally rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-7 and 12-15 of USSN 10/855,755. Applicants traverse.

Claims 1-7 and 12-15 of USSN 10/855,75 are not currently pending. Applicants submit that the provisional rejection of claims 1, 3, 10, 13 and 14 over these non-pending claims is moot, and respectfully request that it be withdrawn.

## CONCLUSION

In view of the foregoing, Applicants believes all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the claims are deemed not to be in condition for allowance after consideration of this Response, **a telephone interview with the Examiner is hereby requested prior to preparation of an additional Office Action**. Please telephone the undersigned at (510) 337-7871 extension 235 to schedule an interview.

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Attachments:

1) A petition to extend the period of response for **1** month;
2) A transmittal sheet;
3) A fee transmittal sheet;
4) Supplemental IDS and Form 1449 and, reference; and
5) A receipt indication postcard.

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